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A Mouse Peritonitis Model for the Study of Glycopeptide Efficacy in GISA Infections

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ABSTRACT

In recent years, the emergence of *Staphylococcus aureus* strains with reduced susceptibility to glycopeptides has raised considerable concern. We studied the efficacy of vancomycin and teicoplanin, as well as cloxacillin and cefotaxime, against the infection caused by four *S. aureus* strains with different glycopeptide and β-lactam susceptibilities (strains A, B, C, and D; MICs for vancomycin of 1, 2, 4, and 8 μg/ml respectively), using a modified model of mouse peritonitis. This optimized model appeared to be straightforward and reproducible, and was able to detect low differences in bacterial killing between antibiotics and also between different *S. aureus* strains. Bactericidal activities in peritoneal fluid for vancomycin, teicoplanin, cloxacillin, and cefotaxime decreased from -2.98, -2.36, -3.22, and -3.57 $\log_{10}$ cfu/ml, respectively, in infection by strain A (MICs for vancomycin and cloxacillin of 1 and 0.38 μg/ml, respectively) to -1.22, -0.65, -1.04, and $+0.24$ in peritonitis due to strain D (MICs for vancomycin and cloxacillin of 8 and 1,024 μg/ml). Our data confirm the superiority of β-lactams against methicillin-susceptible *S. aureus* and show that bactericidal activity of glycopeptides decreases significantly with slight increases in MICs; this finding suggests a reduced efficacy of glycopeptides in the treatment of serious glycopeptide-intermediate *S. aureus* infections.

INTRODUCTION

GLYCOPEPTIDES ARE CONSIDERED the antibiotics of choice for the treatment of moderate to severe methicillin-resistant *Staphylococcus aureus* (MRSA) infections. However, the emergence of *S. aureus* strains with decreased susceptibility to these antibiotics is a matter of concern.[2,3,9,10,12,20,29,35,36] Although the clinical relevance of glycopeptide-intermediate *S. aureus* (GISA) is controversial due to the lack of controlled studies, the poor outcome obtained using vancomycin in some difficult-to-treat infections such as endocarditis or orthopedic surgical infections[1,5] suggests that glycopeptide therapy may be suboptimal in this setting. To date, experimental studies assessing the best treatment of GISA infections have been scarce and inconclusive.[5] Rabbit endocarditis or foreign body infection in rats or mice have been mostly used to study *S. aureus* infections and alternative therapies. These models closely simulate the characteristics of the infection in humans and provide clear end points that allow statistical comparisons between different therapeutic regimens, but their implementation is complex and they involve a considerable investment of time and money. To evaluate effortlessly the *in vivo* efficacy of different antimicrobial treatments for MRSA and GISA infections, we have developed a modified model of mouse peritonitis, using clinical strains of *S. aureus* with different susceptibilities to β-lactams and glycopeptides. The mouse peritonitis model is straightforward, rapid, and easily reproducible, and has been widely used in testing antibiotics *in vivo*[8,11,13–16,18,23,26]; however, although it has been used for the study of *S. aureus* infections,[11,16,18,23,26] only a few comparative therapeutic studies have been reported. In this particular study, we performed the standardization of the model and assessed the comparative efficacy of monotherapy with cloxacillin, cefotaxime, vancomycin, and teicoplanin against the infection caused by four *S. aureus* strains.

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MATERIALS AND METHODS

Bacterial strains

Four staphylococcal clinical strains with different susceptibilities to glycopeptides and β-lactams were studied. Strains HUB 954 (strain A), HUB 284 (strain B), and HUB 783 (strain C) were isolated in our hospital and strain Mu 50 (ATCC 700699) (strain D) was isolated in Japan in 1997 and described as the first GISA strain.[10] Strain C, belonging to the Iberian clone, had heterogeneous resistance to vancomycin (HRV), growing on 4 μg/ml Müeller–Hinton plates with a frequency of subpopulations of 3.6×10^{-6} cfu/ml[1]. MICs of strains A, B, C, and D were determined by the standard macrodilution method.[24] Values of MICs are shown in Table 1.

Inoculum preparation

Colonies from fresh overnight cultures on 5% blood agar plates were resuspended and grown for 4–6 hr at 37°C in TSB medium. Immediately before inoculation, cultures in TSB were centrifuged and resuspended in sterile saline, adjusted to an optical density equal to 0.5 McFarland ($\sim 10^8$ cfu/ml) and then diluted to the appropriate size. Inoculum sizes from 10^6 to 10^8 cfu/ml were tested.

Mouse peritonitis model

The animal studies were approved by the Ethical Committee for Animal Experiments at the University of Barcelona. The mouse peritonitis model was a modified version of a previously described protocol.[8,13,14] Two different mouse strains were tested: Outbred, female, ICR CD-1 mice (~8 weeks; ~30 grams) and inbred, female, C57BL/6 mice (~6 weeks; ~14–16 grams). Mice were kept 10 to a cage and had food and water *ad libitum.* Inoculation was performed by intraperitoneal (i.p.) injection of 0.5 ml of the inoculum with a 26-gauge syringe. The inoculum consisted of a staphylococcal suspension with 5% (wt/vol) mucin in sterile saline, which enhances the basal virulence of bacteria by inhibiting the local macrophage system, and renders the mice susceptible to infection. In the first stage of the study, the experimental design was standardized, prior to the pharmacokinetic and antibiotic therapy studies. Mouse strain, inoculum size, and virulence of different bacterial strains were evaluated.

TABLE 1. MICs (μG/ML) OF *S. AUREUS* STRAINS USED IN THE STUDY

	MIC (μg/ml)[a]			
Antibiotic	*Strain A (MSSA)*	*Strain B (MRSA)*	*Strain C (HRV)*	*Strain D (GISA)*
Cloxacillin	0.38	512	1,024	1,024
Cefotaxime	0.5	1,024	1,024	2,048
Vancomycin	1	2	4	8
Teicoplanin	0.5	1	8	8

[a]MSSA, Methicillin-susceptible *S. aureus*; MRSA, methicillin-resistant *S. aureus*; HRV, heterogeneous resistance to vancomycin; GISA, glycopeptide-intermediate *S. aureus*.

Different time points were tested to study the evolution of the infection and appropriate time points were set for starting therapy. Groups of mice were killed at 4, 8, 16, and 24 hr postinoculation to obtain blood and peritoneal fluid (PF) samples as described below. Bacterial counts and bacteremia were determined for each mouse and time point. Mortality was also assessed.

Sample collecting and processing

At each time point, for all experiments, mice were anesthetized i.p. with 40 μl of ketamine/xylazine 10:1 (100 mg/kg Ketamine and 10 mg/kg Xylacine) and a peritoneal wash was performed by injecting 2 ml of sterile saline i.p. followed by a 1-min external massage of the abdomen. Immediately, 0.1 ml of blood was withdrawn by cardiac puncture, and animals were then killed by cervical dislocation. The abdomen was opened and 0.2 ml of PF was recovered from the peritoneum using an aseptic technique. PF samples were used to perform direct and 10-fold diluted cultures, which were plated (0.1 ml) on 5% sheep blood TSA plates and incubated for 24 hr at 37°C to determine bacterial counts. The detection limit using this method was 10^1 cfu/ml; a value of 0.9 log cfu/ml was assigned to the first sterile culture and 0 to the subsequent ones. To avoid carryover antimicrobial agent interference, the sample was placed on the plate in a single streak down the center and allowed to absorb into the agar until the plate surface appeared dry; the inoculum was then spread over the plate. Plates were incubated overnight at 37°C. Blood cultures were also performed. Immediately after cardiac puncture, blood samples (100 μl) were cultured in Trypticase soy broth for 24 hr at 37°C, and then 100 μl of the cultured broth were plated in TSA plates and incubated again for 24 hr to assess bacterial growth. Bacteremia was expressed on the basis of blood cultures. Mortality was also expressed qualitatively.

Pharmacokinetics

Pharmacokinetic studies were performed to select dose regimens that result in serum concentrations similar to those in humans. All antimicrobials were administered subcutaneously. Groups of 20–22 healthy mice were used for each antibiotic pharmacokinetic study. A single weight-adjusted dose of the antibiotic was administered to each animal, following previous pharmacokinetic experimental studies.[15,19,22,27,28,31,37,38]

After antibiotic administration and at different time points, sets of 3 animals were anesthetized i.p. with appropriate doses of ketamine/xylacine, and blood samples (~400–500 μl) were obtained by an incision in the periorbital plexus of the eye. Blood was centrifuged and serum stored at −80°C until analysis. Pharmacokinetic and pharmacodynamic (PK and PD) parameters were obtained by a computer-assisted method (PK Functions for Microsoft Excel. J. I. Usansky, A. Desai, and D. Tang-Liu, Department of Pharmacokinetics and Drug Metabolism, Allergan, Irvine, CA 92606) after determination of antibiotic concentration at the different time points. The parameters calculated were: peak drug concentration in serum (C_{max}), elimination half-life ($T_{1/2}$), area under the concentration–time curve (AUC), inhibitory quotient (IQ; IQ = C_{max}/MIC), and time above the MIC of the drug concentration in serum (T > MIC). Based on previous studies,[6,15,16] human data,[7,21,30] and

the PK and PD parameters obtained, doses administered to mice were finally selected: vancomycin 30 mg/kg per 4 hr (daily dose of 180 mg/kg), teicoplanin 40 mg/kg per 24 hr (daily dose of 40 mg/kg), cloxacillin 160 mg/kg per 2 hr (daily dose of 1.6 g/kg), and cefotaxime 200 mg/kg per 2 hr (daily dose of 2 g/kg).

Therapeutic experiments

Once the experimental design of the animal model was finalized, therapeutic experiments were performed. Four hours after inoculation, antibiotic therapy was initiated (hour 0). Vancomycin, teicoplanin, cloxacillin, or cefotaxime were administered as single regimens using the antibiotic schedule described above. At hour 0, 2 mice were killed and used as controls. Groups of 8 mice were then randomized to different therapeutic regimens ($n = 6$) and control group ($n = 2$). Antibiotic treatment or placebo was administered for 24 hr and mice were killed at that time point to obtain blood and PF samples. Therapeutic experiments were repeated several times to achieve a minimum number of animals in all groups that allowed statistical analysis.

Antibiotic assays

Antibiotic assays were performed in duplicate. The serum concentrations of vancomycin and teicoplanin were determined by fluorescent polarization immunoassay (FPIA) using a TDx analyzer (ABBOTT CIENTÍFICA, S.A., Diagnostics Division, Costa Brava 13, 28034 Madrid, Spain); minimal detectable concentrations were 2.0 μg/ml for vancomycin and 1.7 μg/ml for teicoplanin. Serum concentrations of cloxacillin and cefotaxime were determined by the disk diffusion bioassay method,[4] using *S. aureus* ATCC 29213 and *E. coli* ATCC 25922, respectively, as test organisms. The minimal detectable concentration was 0.5 μg/ml for cloxacillin and 1 μg/ml for cefotaxime.

Statistical analysis

All bacterial count data were checked for normal distribution (Kolmogorov–Smirnov test). Analysis of variance (ANOVA) with the Scheffé *post hoc* test was used to analyze multiple comparisons among therapeutic and control groups in every strain. Also comparisons among strains were made by using ANOVA (with Tukey *post hoc* test). The two-tailed Fisher's exact test was used for categorical data (survival, bacteremia). A linear regression was performed to assess correlation between *in vivo* vancomycin efficacy (bacterial count) and vancomycin susceptibility of the four strains (MICs).

RESULTS

Standardization of the model

An inoculum of 10^6 cfu/ml of *S. aureus* strain A was used to compare mouse strains. The results of the immunocompetent inbred C57BL/6 mouse strain were more homogeneous than those of its outbred counterpart ICR CD-1. Dispersion in bacterial count data obtained at 4 and 24 hr post-inoculation was significantly higher for ICR CD-1, whereas the mean bacterial count was higher for C57BL/6 (Fig. 1A). Bacteremia was higher in the C57BL/6 strain: 100% at 4 and 24 hr post-inoculation for C57BL/6 and 71% and 80% at 4 and 24 hr, respectively, for ICR CD-1. Mortality at 24 hr post-inoculation was 68% ($n = 19$) for C57BL/6 and 25% ($n = 8$) for ICR CD-1. Inoculums from 10^6 to 10^8 cfu/ml of *S. aureus* strains A and C were compared in the C57BL/6 mice. The 10^8 cfu/ml inoculum was rejected because mortality was 100% less than 16 hr post-inoculation. Bacterial concentration at 4, 8, and 24 hr post-inoculation did not show differences between 10^6 and 10^7 inoculums or between bacterial strains A and C. Bacteremia at 24 hr post-inoculation was lower in mice infected with a 10^6 cfu/ml inoculum (72%) than in those infected with a 10^7 cfu/ml inoculum (100%, as mentioned above). Mortality was the parameter that varied most according to inoculum size: 8 hr post-inoculation, it was 72% ($n = 22$) with the 10^7 cfu/ml inoculum, but zero ($n = 8$) with the 10^6 cfu/ml inoculum. At 24 hr post-inoculation, mortality was 100% ($n = 13$) and 68% ($n = 19$) for 10^7 and 10^6 cfu/ml inoculums, respectively (Fig. 1B). So the final experimental design for therapeutic studies included C57BL/6 mice and inoculum sizes of 5×10^6 to 5×10^7 cfu/ml depending on bacterial strain. Therapy was initiated 4 hr post-inoculation to avoid early mortality.

Pharmacokinetics

Pharmacokinetic and pharmacodynamic parameters of antibiotics used in the experiments are shown in Table 2. $T_{1/2}$ obtained for β-lactams in mice is extremely short, and so the interdose time for these antibiotics was also very short. Although the serum peak was very high for cefotaxime and cloxacillin, $T >$ MIC for strains B, C, and D was 0. Only for strain A did the $T >$ MIC of β-lactams reach 90–100%. Vancomycin showed a high serum peak and $T >$ MIC ranging from 45% to 90% depending on the strain. Finally, teicoplanin achieved a very high AUC (>700 μg/hr per ml) due to its high serum peak and long $T_{1/2}$.

Antibiotic efficacy: bacterial clearance in peritoneal fluid, bacteremia, and mortality

Initial bacterial counts in PF (mean $\pm$ SD), corresponding to hour 0 of the control group for the different strains were: 7.62 ± 0.37 ($n = 23$) for strain A, 7.05 ± 0.43 ($n = 15$) for strain B, 7.04 ± 0.55 ($n = 28$) for strain C, and 7.02 ± 0.35 ($n = 13$) for strain D. Bacteremia in control animals at 0 and 24 hr, expressed as percentage of positive blood cultures, was 100% for all strains. Mortality of control mice at 24 hr differed according to strain: 55% for strain A, 22% for strain B, 62% for strain C, and 21% for strain D.

Bacterial clearance in PF after 24 hr therapy for strains A, B, C, and D is shown in Fig. 2; $n = 6$ for all therapeutic groups in all strains and $n \geq 12$ for 24-hr control groups of all strains. In strain A peritonitis, cloxacillin and cefotaxime were bactericidal at 24 hr. Vancomycin and teicoplanin showed slightly lower activity than β-lactams, although the difference was not significant. All therapies had significantly lower bacterial counts than the control group. All treated animals survived. Bacteremia at 24 hr for cloxacillin and cefotaxime treatments was 0%, but 25% for vancomycin and 100% for teicoplanin.

For strain B, vancomycin and teicoplanin achieved a significantly higher reduction in bacterial count than β-lactams, in which the decrease was minimal. There was no statistically sig-

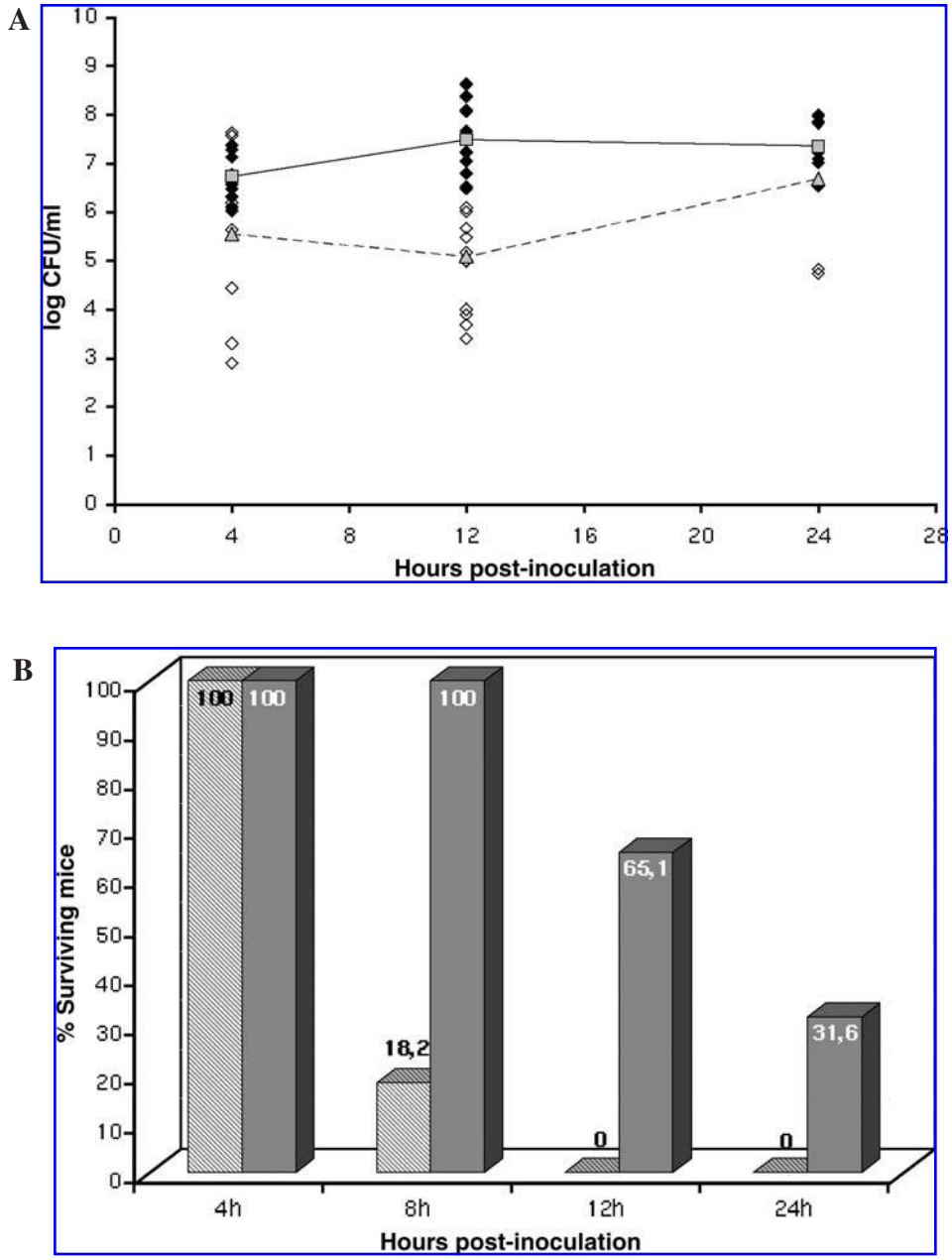

FIG. 1. (**A**) Comparison of data dispersion of bacterial count in PF between ICR (open symbols) and C57BL/6 (solid symbols) mouse strains at different time points. Each symbol represents one mouse. Solid squares and triangles linked by lines represent the respective means of C57BL/6 and ICR. (**B**) Survival at 4, 8, 12, and 24 hr post-inoculation of C57BL/6 mice with peritonitis due to *S. aureus*. Comparison between 10^7 (hatched columns) and 10^6 (gray columns) cfu/ml inoculums.

nificant difference between the β-lactam and the control group. No mortality was observed in any treatment group. Vancomycin reduced bacteremia at 24 hr to zero and teicoplanin to 50%. Bacteremia fell to 80% with cefotaxime, but remained at 100% at 24 hr with cloxacillin.

In strain C experiments, only vancomycin was able to reduce bacterial counts that differed significantly from those of the control group. It also differed significantly from cefotaxime, which showed an increase in bacterial count at 24 hr, and was totally ineffective. Mortality for cefotaxime-treated mice was 66%, whereas survival was 100% in the other treatment groups. Cloxacillin and cefotaxime were not able to reduce bacteremia. Teicoplanin decreased bacteremia slightly to 83% and vancomycin reduced it to 33%.

Finally, in strain D experiments, vancomycin showed only moderate activity, lower than that achieved in strain C. Teicoplanin reduced the bacterial count slightly, showing lower activity than cloxacillin, and less activity also than that achieved with strain C. The activity of cloxacillin was slightly higher than that obtained for strains B and C. All treated animals sur-

TABLE 2. PHARMACOKINETIC AND PHARMACODYNAMIC PARAMETERS OF ANTIBIOTICS USED IN THE EXPERIMENTS

Parameter	*Antibiotics*			
	Cloxacillin	*Cefotaxime*	*Vancomycin*	*Teicoplanin*
Dose	160 mg/kg	200 mg/kg	30 mg/kg	40 mg/kg
C_{max} (μg/ml)	163.5	162.3	44	148
$t_{1/2}$ (hr)	0.35	0.19	0.56	3.26
AUC (μg/hr per ml)	116.25	76.4	45.4	729
IQ (C_{max}/MIC)				
Strain A	430.13	324.68	44.01	296.20
Strain B	<0.64	<0.63	22.01	148.10
Strain C	<0.64	<0.63	11.00	18.51
Strain D	<0.64	<0.63	5.50	18.51
$t >$ MIC (hr) [%]				
Strain A	3.4 [>100]	1.78 [~80]	3.47 [87]	25.94 [>100]
Strain B	0 [--]	0 [--]	2.91 [73]	22.68 [95]
Strain C	0 [--]	0 [--]	2.35 [59]	12.91 [54]
Strain D	0 [--]	0 [--]	1.79 [48]	12.91 [54]

vived, except those treated with cefotaxime; cefotaxime was ineffective, and presented a mortality of 14% at 24 hr. Vancomycin and cloxacillin reduced bacteremia to 28.6%, whereas teicoplanin and cefotaxime achieved no reduction.

Comparison of glycopeptide therapy between the four strains in the study is shown in Fig. 3; its efficacy was significantly lower with infections due to strains C and D (HRV, GISA) than with infections due to strains A and B.

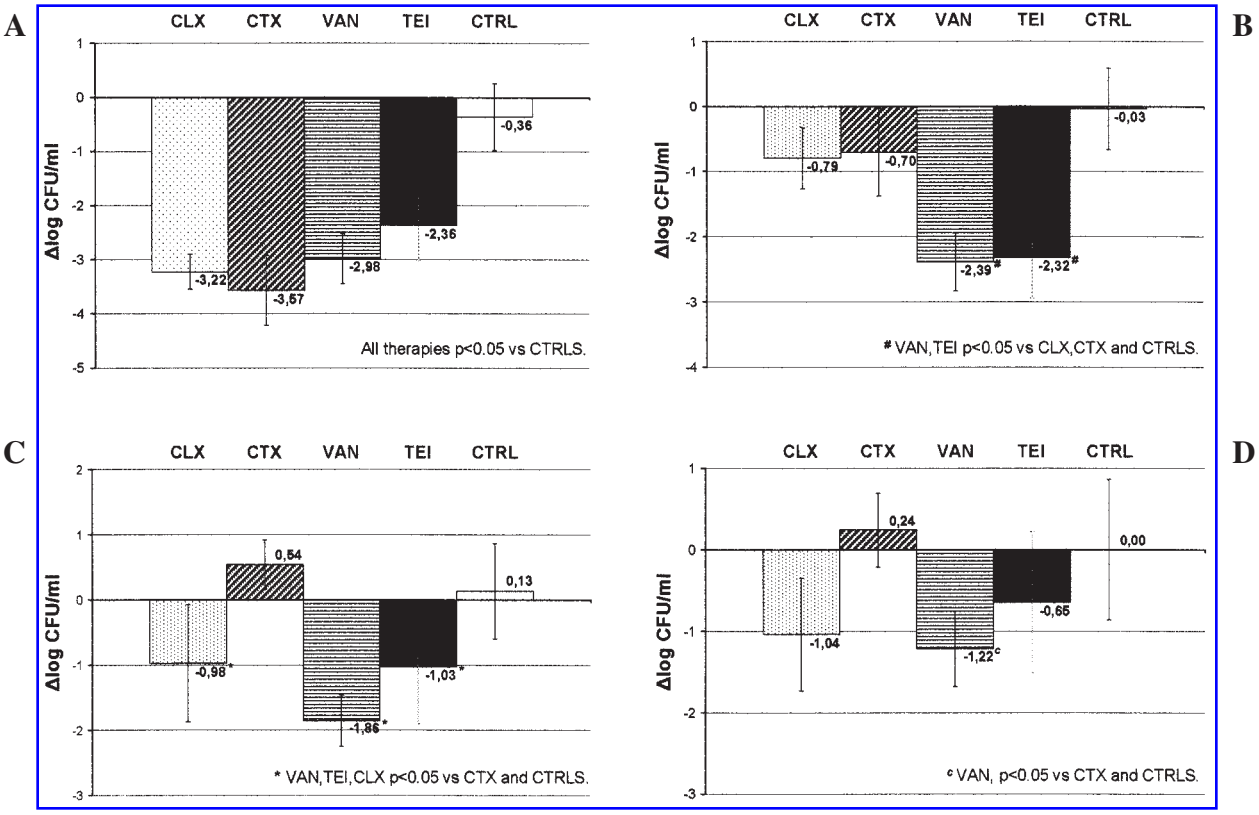

FIG. 2. Bacterial killing rates in peritoneal fluid after 24 hr of therapy for strains A (**A**), B (**B**), C (**C**), and D (**D**). Results are expressed as differences in mean bacterial counts between the different groups at 24 hr of treatment and controls at 0 hr ($\Delta\log_{10}$ cfu/ml (24–0 hr). Mean bacterial counts ± SD at 24 hr for the different strains were, for strain A: CLX 4.40 ± 0.32, CTX 4.05 ± 0.65, VAN 4.64 ± 0.46, TEI 5.26 ± 0.65, and Control (CTRL) 6.95 ± 0.62; for strain B: CLX 6.26 ± 0.47, CTX 6.35 ± 0.67, VAN 4.66 ± 0.44, TEI 4.73 ± 0.63, and Control (CTRL) 7.01 ± 0.62; for strain C: CLX 6.08 ± 0.90, CTX 7.59 ± 0.38, VAN 5.19 ± 0.39, TEI 6.02 ± 0.88, and Control (CTRL) 7.18 ± 0.73; and for strain D: CLX 5.98 ± 0.69, CTX 7.26 ± 0.45, VAN 5.80 ± 0.46, TEI 6.37 ± 0.87, and Control (CTRL) 7.02 ± 0.75. $n = 6$ for all therapeutic groups and $n \geq 12$ for control groups at 24 hr.

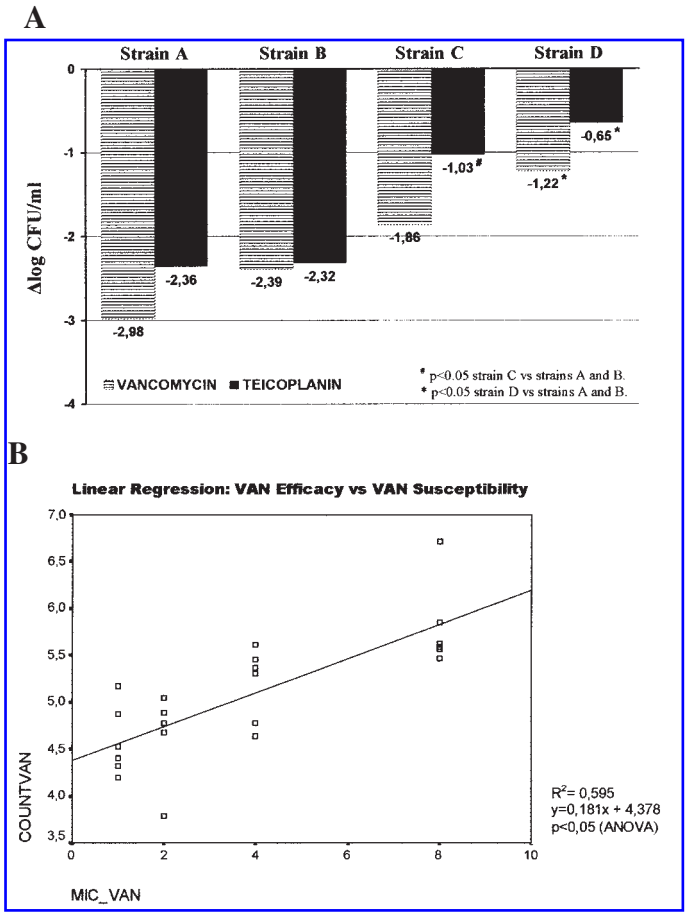

FIG. 3. (**A**) Comparison of vancomycin and teicoplanin bacterial killing rates at 24 hr among *S. aureus* strains A, B, C, and D. Results are expressed as $\Delta\log_{10}$ cfu/ml (24–0 hr). Mean bacterial counts ± SD at 24 hr for the different strains were: strain A, VAN 4.64 ± 0.46, TEI 5.26 ± 0.65; strain B, VAN 4.66 ± 0.44, TEI 4.73 ± 0.63; strain C, VAN 5.19 ± 0.39, TEI 6.02 ± 0.88; and strain D, VAN 5.80 ± 0.46, TEI 6.37 ± 0.87. $n = 6$ for all groups. (**B**) Linear regression between vancomycin susceptibility, expressed as the MIC of the different strains (x axis) and vancomycin efficacy, expressed as bacterial counts (log cfu/ml) of vancomycin-treated groups (y axis). Each point represents one mouse.

DISCUSSION

In general, previous experimental studies with the *S. aureus* mouse peritonitis model have not focused on comparison of different antibiotic regimens. We developed a modified version of the model previously described, evaluating variables involved in the experimental design to obtain a model that was sufficiently sensitive for detecting differences in efficacy among different antibiotic regimens. Results for mean bacterial count, bacterial count dispersion, and bacteremia suggested that the C57BL/6 mouse strain performed better, showing more susceptibility to infection and also providing more homogeneous findings. Among the different parameters used, the bacterial count of peritoneal fluid was the most useful to assess antibiotic efficacy, whereas survival was less sensitive for comparisons between different antibiotics in this model. Therefore, we sought an inoculum size that could produce a high bacterial count in peritoneal fluid, high bacteremia, and low mortality during the first hours after inoculation. While bacterial counts in peritoneal fluid of 7–8 $\log_{10}$ cfu/ml and 100% bacteremia were found using inoculums of both 10^6 and 10^7 cfu/ml, mortalities ranged from 21% to 62%, depending on the strain, reflecting the virulence of the model. We therefore used 5–9 $\times$ 10^6 as the inoculum size of choice for strain A and 1–5 $\times$ 10^7 cfu/ml for strains B, C, and D to obtain a similar mortality rate. The mouse peritonitis model was highly reproducible, and it was easier to perform and cheaper than other models used in experimental *S. aureus*-related infections.

Antibiotic dosing regimens were chosen to obtain appropriate PK/PD parameters that simulate those in humans.[6,7,21] Vancomycin at a dose of 30 mg/kg reached a good peak in mice serum (within the range of that in humans), with high IQ values for strains A and B, within the recommended values for strain C and slightly below that value for strain D. This dose was administered every 4 hr (final dose of 180 mg/kg per day) to maintain a good $T >$ MIC value, which was high for strains A and B and below 50% of interdose time for strains C and D. Efficacy of vancomycin decreased from strain A to D, showing a good correlation with the MICs of the different strains and the PK/PD parameters obtained. Teicoplanin at 40 mg/kg achieved a serum peak and trough similar to those in humans and, as expected from its long elimination half-life, showed a very high AUC. It could therefore be administered in a single daily dose. Higher IQs than vancomycin, (minimal ~10 for strain D) and $T >$ MIC from >100% for strain A to ~40% for strain D confirmed an optimal PK/PD profile, similar to that in humans.

Teicoplanin showed good efficacy for strains A and B, but only moderate for strains C and D, in accordance with its pharmacodynamic profile, but with some differences with respect to vancomycin. Vancomycin showed slightly improved activity than teicoplanin in experiments with strains A and B; this better efficacy is more evident for strains C and D although no significant differences were found between the two therapies. Thus, teicoplanin presented better PK/PD profiles than vancomycin, but it was not more effective *in vivo,* as others have already reported.[27,38] It is interesting how this point becomes more apparent with GISA strains, in which teicoplanin therapy was clearly less effective than vancomycin. Recently, Pavie *et al.* have reported the impact of the GISA phenotype when using teicoplanin in therapy for experimental endocarditis,[25] an observation that is in agreement with our results. High levels of protein binding and low penetration in some tissues have been proposed as possible causes for teicoplanin failure in some clinical and experimental infections.[15,16,25]

Cloxacillin and cefotaxime showed bactericidal efficacy in the treatment of peritonitis due to strain A, as was expected from their PK/PD profiles. On the other hand, vancomycin and teicoplanin showed good activity but were not bactericidal at 24 hr. Our results corroborate previous reports that glycopeptides are less bactericidal than β-lactams in the treatment of serious infections such as endocarditis.[5,17,32] Cefotaxime and cloxacillin preserved a slight activity against strain B despite the fact that their $T >$ MIC was 0, and, more surprisingly, cloxacillin retained a moderate activity against strains C and D, a finding that was not consistent with its pharmacodynamic pa-

rameters. A partial explanation for this observation may be the reciprocal loss of resistance to oxacillin in some strains as vancomycin MICs increase, as was previously described by Sieradzki and Tomasz.[33,34] On the one hand, they noted that vancomycin-resistant *S. aureus* laboratory-derived mutants reduced β-lactam resistance as increased MICs of the glycopeptides; on the other, they reported that the acquisition of this resistance to vancomycin involves the change from the homogeneous to the heterogeneous methicillin-resistant phenotype. Thus, although hGISA and GISA strains used in our experiment were still highly resistant to methicillin on the basis of their MICs, we can hypothesize that the increasing *in vivo* efficacy of cloxacillin may be due to the emergence of subpopulations less resistant to methicillin, reflecting an early stage in the change from the homogeneous to the heterogeneous methicillin-resistant phenotype.

The efficacy of vancomycin and teicoplanin to treat peritonitis due to strains B, C, and D decreased progressively. Interestingly, percentages of bacteremia increased as killing rates achieved by glycopeptides decreased. Comparison of glycopeptide efficacy among the four strains showed that bactericidal activity decreased significantly with slight increases in glycopeptide MICs (Fig. 3A). The linear regression shown in Fig. 3B underscores this inverse linear relationship between vancomycin MIC and its efficacy *in vivo*. This result suggests that any decrease in glycopeptide susceptibility may have clinical implications, especially in the setting of difficult-to-treat infections such as endocarditis or orthopedic infection, as has been previously described.[1,5] On the other hand, vancomycin keeps significant activity compared to controls in strains C and D (Fig. 3D). Although scarce, this remnant bacteriostatic activity could be relevant in the clinical setting, in which vancomycin can be still useful in relatively mild infections such as nonnecrotizing skin and soft tissue infections, but not in severe infections.

In conclusion, this mouse peritonitis model is straightforward and reproducible. It is able to detect low differences in bacterial killing among antibiotics and also among *S. aureus* strains with different methicillin and glycopeptide susceptibilities. Therefore, this model is particularly useful for comparing the efficacy of antibiotics against these strains. Although the results obtained with experimental infections must be interpreted with caution, our study suggests that heterogeneous resistance to glycopeptides and increases in glycopeptide MICs may be associated with a loss of efficacy in these antibiotics. These findings help to explain previous reports of therapeutical failures with infections due to HRV and GISA strains. This experimental model seems particularly well suited to the task of finding alternative treatments for these infections.

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